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硕 士 学 位 论 文

氨基胍对缺血再灌注肾损伤小鼠的保护作用及可能机制

The renoprotective effects and possible mechanism of aminoguanidine in mice with ischemia/reperfusion injury

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摘 要

缺血再灌注损伤（ischemic reperfusion injury, IRI)是指组织或器官在缺血及重获血流灌注或氧供应后，对组织或器官产生的损伤作用。肾是高灌注器官，对缺血缺氧较为敏感，因此肾缺血—再灌注损伤在临床上较为常见。研究表明，肾缺血—再灌注损伤是引起急性肾衰竭（acute renal failure，ARF）和慢性肾衰竭（chronic renal failure，CRF）的主要原因。但是缺血再灌注肾损伤的发病机制至今尚未完全阐明。因此，明确肾缺血—再灌注损伤的发病机理，寻求缓解损伤或者治疗方法成为当前研究的热点。

研究表明缺血再灌注肾损伤的发生与 ATP 减少、大量氧自由基(Oxygen free radical，OFR)产生、细胞内钙超载和细胞凋亡基因调控等介导的肾小管和肾小球细胞损伤的关系密切。亦有研究显示诱导型一氧化氮合酶（inducible nitric oxide synthase，iNOS）在肾缺血再灌注损伤中发挥重要作用，但其具体作用目前尚存在争议。有研究表明，降低 iNOS 表达可以减轻缺血再灌注损伤，而另一些研究则认为提高 iNOS 表达能减轻缺血再灌注损伤。为进一步探讨肾缺血再灌注损伤与 iNOS 的关系，我们构建了小鼠缺血再灌注肾损伤模型，通过 real-time PCR 和免疫组织化学方法对比 iNOS mRNA 和蛋白在假手术组和缺血再灌注组小鼠表达的差异，同时给予缺血再灌注肾损伤小鼠术前应用 iNOS 特异性抑制剂 AG 并对比肾损伤程度，以期明确肾缺血再灌注损伤和 iNOS 的关系并寻求治疗方法。本研究主要由以下三部分内容组成。

一、小鼠缺血再灌注肾损伤模型的建立

为研究缺血再灌注肾损伤的发生机理，我们制作了小鼠缺血再灌注肾损伤模型。制作肾 IRI 动物模型的方法包括双侧肾蒂夹闭、双侧肾动脉夹闭、单侧肾切除+对侧肾动脉夹闭等。本实验是通过双侧肾蒂夹闭法制作小鼠缺血再灌注模型。通过参阅相关文献及多次预实验，我们发现手术方法不同、血管夹闭的时间长短、术中温度的控制、鼠龄等均对实验结果有重要影响，尤其是术中温度和夹闭肾蒂的时间在此过程中起着不可忽视的作用。经过不断摸索，多次检测造模 24 小时后尿素氮、肌酐水平，我们最终确定术中温度 37℃，双侧肾蒂夹闭 45 分钟为制

作本模型的必需条件。

二、小鼠缺血再灌注肾损伤对iNOS的影响

一氧化氮合酶（nitric oxide synthase，NOS）是一氧化氮（oxide synthase，NO）生物合成的关键限速酶，可催化 L-精氨酸转化为 L-胍氨酸和 NO。主要包括神经元型 NOS、内皮型 NOS 和 iNOS。病理状态下，iNOS 可被细胞因子、创伤等诱导表达并催化产生大量 NO，引起体内一系列病理生理变化，导致组织器官损伤。但是 iNOS 在缺血再灌注损伤中的作用一直存在争议。本研究通过建立小鼠缺血再灌注肾损伤模型，造模 24 小时后提取小鼠肾组织 mRNA 进行 real-time PCR 检测 iNOS mRNA 的变化情况并通过免疫组织化学技术对比小鼠缺血再灌注肾损伤后 iNOS 蛋白表达水平的变化情况，结果发现缺血再灌注肾损伤后小鼠 iNOS mRNA 和蛋白的表达均明显增加，提示 iNOS 增高可能是引起小鼠缺血再灌注肾损伤的发病机理之一。

三、 氨基胍（aminoguanidine，AG）对缺血再灌注肾损伤的保护作用及其机制

氨基胍是一类胍类化合物，可特异性抑制 iNOS 活性，减少 NO 生成。本研究发现术前注射 AG（50mg/Kg）可明显降低缺血再灌注肾损伤后的 iNOS mRNA 和蛋白的表达水平，减轻肾损伤程度（尿素氮、肌酐水平降低）。究其原因可能是 AG 特异性抑制 iNOS 的表达，减少其催化产生的 NO 及氧自由基，从而阻断了 NO 及氧自由基等所介导的细胞毒效应，发挥保护肾组织的作用。

总之，我们通过以上三部分研究得到以下结论：（1）术中温度为 37℃，双侧肾脏缺血 45 分钟再灌注，24 小时后小鼠尿素氮、肌酐水平明显升高，达到急性肾衰竭，是小鼠缺血再灌注急性肾损伤模型建立的必要条件；（2）AG 可降低缺血再灌注急性肾损伤小鼠尿素氮、肌酐水平，保护肾功能；（3）缺血再灌注急性肾损伤小鼠 iNOS mRNA 和蛋白表达水平明显升高，提示缺血再灌注肾损伤可能与 iNOS 水平升高相关；（4）AG 可以通过特异性抑制 iNOS 发挥肾脏保护作用。

缺血再灌注肾损伤是急性肾衰竭的主要原因。损伤后不完全修复及纤维组织增生可引起持续性肾脏损坏并逐渐进展至慢性肾衰竭，严重危害国人健康，目前尚不明确其发病机制且无特效治疗方法。本研究发现缺血再灌注肾损伤可引起 iNOS 明显升高，加重肾损伤，并发现应用 iNOS 抑制剂 AG 可明显减轻肾损伤，提示缺血再灌注肾损伤与 iNOS 升高密切相关且 AG 具有治疗缺血再灌注肾损伤

的作用，为缺血再灌注肾损伤的治疗提供了有力依据。

关键词：氨基胍　缺血再灌注肾损伤　诱导型一氧化氮合酶

Abstract

Ischemia-reperfusion injury (IRI) is the damage that caused by ischemia and perfusion or oxygen supplied again on the tissue or organ. Kidney is a high perfused organ, it's more sensitive to ischemia and hypoxia, so renal ischemia - reperfusion injury is very common in clinical. Studies have shown that renal ischemia - reperfusion injury is the main reason that caused acute renal failure (acute renal failure, ARF) and chronic renal failure (chronic renal failure, CRF). But pathogenesis of ischemia-reperfusion renal injury has not yet fully understood. Therefore, expore the pathogenesis of renal ischemia - reperfusion injury, seeking relief or treatment of it become a research focus.

Existing research data indicate that renal ischemia-reperfusion injury had relationships with reduced the occurrence of ATP, generation of a large number of oxygen free radicals (OFR), intracellular calcium overloaded and gene regulation of apoptosis which mediated by renal tubular and glomerular cell injury . Studies have shown that inducible nitric oxide synthase (iNOS) play an important role in renal ischemia reperfusion injury, but its specific role is currently still controversial. Some studies have shown that reducing the expression of iNOS can reduce ischemia-reperfusion injury, while other studies have opposed view that increased expression of iNOS can reduce ischemia-reperfusion injury. To investigate the relationgship between the renal ischemia-reperfusion injury and the iNOS level, we producted the renal ischemia-reperfusion injury model，compared the iNOS mRNA and protein by real-time PCR and immunohistochemistry. We hope to know its mechanism and seek the treatment of IRI. This study formed by the following three sections

First, Make the model of renal ischemia-reperfusion injury in mice

To study the mechanism of ischemia-reperfusion injury, we produced a mouse model of ischemia-reperfusion renal injury. The ways to produce animal models of renal IRI including bilateral renal occlusion with pedicle, bilateral renal artery occlusion, unilateral nephrectomy, the contralateral renal artery clipping, etc. In this study, we make the model through the method of bilateral renal pedicle clamping. By referring to literatures and preliminary experiments, we found that the duration

of vascular occlusion, the operation temperature, mouse age and so on have an important impact on the experimental results. Especially the temperature and clipping time played a significant role. After many trials and errors, testing blood urea nitrogen and creatinine levels at 24 hours and 48 hours, we finally determine the conditions to product the model are 37 ℃ opperation temperature and 45 minutes of bilateral renal pedicle clamping.

Second, the impact of ischemia-reperfusion renal injury i on iNOS

Nitric oxide synthase (NOS) is a key rate-limiting enzyme for the biosynthesis of nitric oxide (NO). It can catalyze L-arginine acid changde into L-guanidine acid and NO. NOS includes neuronal NOS, endothelial NOS and iNOS. Under pathological conditions, iNOS could be induced by cytokines, trauma and catalyze to generate a large number of NO and cause a series of pathophysiological changes in the body which can cause the damage of tissue and organ. But the role of iNOS in ischemia-reperfusion injury is controversial. In our study, through the establishment of mouse model of ischemia-reperfusion renal injury, we extract mRNA from mouse renal tissue after 24 hours for real-time PCR to detect the changes of iNOS mRNA and compared iNOS protein expression by immunohistochemistry. we found the expression of iNOS mRNA and protein significantly increased after ischemia-reperfusion renal injury in mice, suggesting the increased expression of iNOS may be the pathogenesis that caused renal injury.

Third, the renoprotective of aminoguanidine (AG) on renal ischemia-reperfusion injury and its mechanism

AG is a class of guanidine compounds, it can specificlly inhibit the activity of iNOS to reduce production of NO. Our study found that preoperative injection of AG (50mg/Kg) can significantly reduce the level of iNOS mRNA and protein after ischemia-reperfusion renal injury, reduce the degree of renal damage (the level of blood urea nitrogen and creatinine decreased.). It may be due to that AG can specificity inhibit the expression of iNOS which can reduce the catalytic NO and oxygen free radicals produced. Because it play a role in kidney protection that reducing the NO and oxygen free radicals which mediate cytotoxicity.

In summary ,We conclued that:1. At 37 degrees, 45 minutes bilateral renal ischemia would cause serious acute renal failure in mice; 2. AG could reduce the Bun and Scr in IRI mice with acute renal failure to keep kidney function; 3. The

level of iNOS mRNA and protein incresed in IRI mice, it may suggest that the severity of renal failure corrlate with iNOS; 4. AG can specificly inhibit iNOS to protect the renal function.

Ischemia-reperfusion renal injury is the main reason of acute kidney failure. Incomplete repair after injury and fibrosis can cause kidney damage sustained and gradually progress to chronic renal failure,.It is serious harm to people's health. We don’t know the pathogenesis and how to treat it till now. Our study found that renal ischemia-reperfusion injury can significantly increase iNOS level and we found the inhibitor of iNOS-AG can significantly reduce the renal injury, suggesting that AG has the effect of treating renal ischemia reperfusion injury.It’s a strong basis for the treatment of renal ischemia reperfusion injury.

Key words: aminoguanidine; ischemia reperfusion injury;
inducible nitric oxide synthase

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